ATTACHMENT 1

Novel levansucrase gene of *Zymomonas mobilis*:

1 gatcttgctt tggcacaggg tgcaaccagt gttgttattg gcggtggtgt cggtcttcgt

61 atcgcttccc atttgccgga atctggcttc cgtcagcgct ttgtttcaaa aggacgcttt

121 gaacgcgtca tgtccaagat tccggttaag ttgattactt atccgcagcc tggactgttg

181 ggtgcggcag ctgcctatgc caacaaatat tctgaagttg aataatattt tttaatatta

241 tgaactgaat ttaagaggct gccttccgat aaaatcggga ggtggccttt tttatatttt

301 ttactaaaaa atgaagacaa aaaactctta agtaagaata atattattat taacttgtga

361 tatattttgt attagttctt cttggtaaag aattattttt gataaatttt gtctaatatc

421 ctatatttta aatatttttt ataatatttt tttaataaaa ttgacgtgat atttaggggt

481 ttgtgtagaa aaatgagata atatttagaa ttattgattt aattctatct agattcatct

541 tagaaattaa tgaatattaa gaagaggcca tcggctattg gagtcgaatt aatatatata

601 caccgtatat catataaacg actatccgac cgaaattata gaaattcgat cgaggatagc

661 cattctaaat taaattatcc tgtatgctct tctactcttt caagaagaac atcgcgttca

721 agataactta cgccagtgcg aatttgacag cagcttctgc atgaatttgt gtggagtcat

781 agcagggcaa atcaagcata tcttgcttga tcagcagagg gatttcagta catccgagga

841 tgacggaatc aacaccttct gcacgggctt tctcaattat cttaagataa ccctgccgtg

901 atgactcttt gacttcaccc tgacaaagtt catcaaaaat cacatcatgg acgaagcctc

961 tatcttcgtc ttccggtgta atcacatcaa gaccctgatt tttcatgcgg tcagtataga

1021 agccatgttc catcgtatag cgcgttgcca agaggagagg gcgtttacga ccatctttct

1081 ttaaggcttt agctgtctca tcaacaatat tgataagagg gacagctgat atctcagcga

1141 cggcatcagc gacgagatgc atcgtgttag tgcaaatgag gacacaatcg gctccagcct

1201 ttgctaagcc tgccccagcg gcaccgagaa gcttggcagc gtcatcccag cggtcagcct

1261 tttgaagcgc aacaacctct gcaaagttca aagaatgcat aagcagatct gctgagacta

1321 atccaccgcg tgcatcacgg atagcttcat tgataatctg gtaataagtg accgtggatt

1381 cccaactcat accgccaata aggccgattt ttttcatatg ccaatctcac gaaactgatt

1441 gttaatagcc ctaagaatgc catgcctttt gtaaaaaaaa cgcgcttttt ttgaggaatt

1501 taagggtaaa actgcaaatt attttcttat tattaggaat aaatgcaaaa atattgttct

1561 tatagaagag tcggtgtatc atttttcgat gatcgactac cgagaccggc atatattatc

1621 tttattacaa gctaacgcag aaatgccatt ggccgagatt gcggaacggg ttgctctatc

1681 tgtttcggct tgttctcgtc gtgtcgcgcg attgcgtgag gaaggctata taaaaggcac

1741 tattgctctt ttggatagaa agaagatcaa tcttccgaca actatttttcc ttcttgtcaa

1801 aacgggattg cacacaggaa attatcttga gcaatttcat gcagccgtga gtgccattcc

1861 tgaaatcgtc gaagtccata ggctcactgg aaattttgat tatattttaa aattagcttt

1921 gccgaatgtc gaatattatg atgttattta taaacagata ctaaaacatg tcgccttcta

1981 tgatatgtct gcctatattt ctatggagac agtaaaaata tcacctgctt taccgacaaa

2041 ttatatttaa aaagaatttt cttctaaaat tttattaaat aattcgtcat cttggctaag

2101 attttttta cctatatcaa tttcttgtaa aaaagcgata atttgatcta tcttattttc

2161 tgaaatttgg gattgttggc tttggtatag taagaaatca agaatagaag gcatgccagc

2221 cgcttgcgct atagcttcaa gatttttgac agacatcacc gaatgatgat tcatatcgac

2281 ggcgcgtgaa agcgttgtcg cggcctttat attagctcta cgtgctaatt ctgcccagct

2341 tacatccatt ttatctcgga tgctgtaaag ccatttggca ataatctctt tttgtctcat

2401 caccttatct cagtaataag tgaggctgaa ggtaatcttt atataaatga gtatataaac

2461 aaaatgttaa attttataaa aatgatattg ttttgttgta ataatattca gaatattaag

2521 gctattggat agataacaag aaatatatta tgaactatca ctcaaaacac ttgggaaggc

2581 caagtggaaa gctaacaaaa cgccgtaaag aaattctgga atattggaga caatatggcc

2641 ctgtctcttt aggaaaattg gcacgtgact gccatatttt tgataggtct acagcaagac

2701 ggtttctaat aacctttgaa aaaatgggta ttttacagcc gcctcgggca aatttttaa

2761 aaaaatagag aaaaaataaa aaatctattt ttatgttgta aattaaaaaa tatacaatga

2821 tttatcaggt taaataattt tttaaaaaac attatattaa aaaatatttt tacttgttgt

2881 aaaataatat ttattaaaat ttatgtaata tttattttaa aattggagat ttatatggtt

2941 gcaaaagatt tgtttttgga attttagtct cttttctcat atatctgaaa tatattgaga

3001 caattcaata ttgataaacc aaacatgttt gggcgttttt ggtataatta ttcaatttat

3061 tttagaatat aaatataaat ataaatataa atttagtttt aagtaaaaac ttccgaaaaa

3121 aatgtgactt cgcttgaaat gtcgaagcgt gtctttcgaa aaggcagcct atttcaatat

3181 ttctgccttt ttgaaagtcg agaacaagaa ttaatctttt ttcggcttta tttcatcatt

3241 tattaggata gttcttatgt tgaataaagc aggcattgca gagccgagct tgtggactcg

3301 tgcggatgct atgaaagtgc ataccgatga tcccacggca accatgccta ccattgatta

3361 tgactttcct gtcatgactg ataaatattg ggtttgggac acttggccct tacgcgatat

3421 taacggtcag gttgtcagct tccaaggttg gtcggtgatc tttgctttgg tcgctgatcg

3481 caccaaatat ggttggcata atcgcaatga tggcgccaga attggttatt tctattcacg

3541 tggtggaagc aactggattt ttggtggtca tcttctgaaa gatggtgcca atccgcgttc

3601 ttgggaatgg tctggttgca cgattatggc accgggtacg gccaattctg tcgaagtatt

3661 ctttacgtct gtcaatgata cgccgtcaga atccgttcct gcccagtgca agggctacat

3721 ctatgccgat gataaatcgg tatggtttga cggttttgat aaagtgaccg atctgtttca

3781 ggcagatggc ctttattatg ctgattatgc agaaaataat ttctgggatt tccgcgatcc

3841 gcatgtcttc attaccccga agataggcaa aacatatgcc ttgtttgaag gtaatgttgc

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4081 ccagacggag cggcctcatg tcgttttcca gaatggcttg acctatctct ttacgatcag

4141 tcatcattcg acttatgccg atggtttgtc gggtcctgat ggggtttatg gctttgtttc

4201 tgaaaacggc atttttggcc cttatgaacc gctgaatggt tccggtttgg ttctcggtaa

4261 cccctcttca cagccttatc aggcttattc ccattatgtg atgacaaatg ggctggtgac

4321 ctccttcatt gataccattc cgagttctga cccgaatgtc tatcgttatg gtggcacctt

4381 ggcaccgacc atcaaattgg aattggttgg ccatcgcagc ttcgttaccg aagtgaaggg

4441 ttatggctat attccgccac agatcgagtg gttggcagaa gatgaatctt ctaattctgc

4501 ggcagccctg tctttattga ataaataaga tttattcatt gtaaatgacg tttctgatta

4561 tgcttgaata aagcattatc atcttttgag tttatttcaa cttaagggtc atcagggcat

4621 tttgccctga tgacaccctt cctattccta ataataattt caagaaagtg tatattactt

4681 taaatgttta attttaatgc cagtcgctgg acgcgagccc aagcgatgaa agtgaataaa

4741 tttgatttga cgacctctat gccggaaatc ggcactgatt ttcccattat gcgtgatgac

4801 ttgtggctgt gggatacttg gccattacgg gatatcaatg gcaatcctgt cagctttaaa

4861 ggctggaatg ttattttctc tttggtcgct gaccgcaata ttccgtggaa tgatcgccat

4921 tctcatgccc gcatcggcta tttctattcc aaagatggta aaagctgggt ttatggtggc

4981 catctattgc aagaatcggc caatacccgc acggcagaat ggtccggcgg cacgattatg

5041 gcaccgggtt cccgtaatca ggtcgaaacc ttctttacct cgactttatt cgacaagaat

5101 ggcgtcagag aagccgttgc tgctgtcacg aaaggccgca tttatgcgga tagtgaaggc

5161 gtttggttca agggttttga ccagtcaacg gatttgtttc aggctgatgg tctgttttat

5221 caaaattatg cagaaaataa tctctggaat ttccgtgatc ctcatgtttt catcaatcct

5281 gaggatggtg agacctatgc tttgttcgaa gccaatgttg ccactgtccg tggagaagac

5341 gatataggcg aggatgaaat tggtcctgtt ccggccaata cggtcgttcc aaaagatgcc

5401 aatttatgct cggcctctat tggtattgcg cgttgtttgt cgccggatcg caccgaatgg

5461 gagctgttac cgcctttgtt gacagccttt ggtgtcaatg accagatgga acggcctcat

5521 gtcattttcc agaatggttt gacctatctc tttacgatca gccatgattc gacttatgcc

5581 gatggcttaa ccggttccga tggtctttat ggctttgttt ccgaaaatgg tatttttggc

5641 ccctatgagc cgctgaatgg ttctggcctt gttctcggtg gccccgcgtc acagccgacc

5701 gaagcctatg ctcattacat catgaataat ggcttggttg aatcttttat caatgaaatc

5761 attgatccca aaagcggcaa agtcattgcg ggcggtagct tggcaccgac ggttcgcgtt

5821 gaattacagg gacatgagac tttcgcaacc gaagtctttg attatggcta tattcccgca

5881 tcttatgctt ggccggtatg gcctttccct gatcgtcgca aataattttt gacgacaaaa

5941 aattgcgctg aaataacgcc aatcataccg gaagatataa aaccggttgt ccattttcag

6001 atgaagtggg agatggtggg cgcggctggg attgaaccag cggccactgc gatgtgaaca

6061 cagtgctcta ccactgagct acgcgcccta tatgctgcga agcagtaaga aaaacccgaa

6121 gctttgtcca gagtttttc aaatagcagg ataaattata aaaatatccc tgccagccgg

6181 tttgactagc agggatacgg ggtaagtgtt ttttatcaat gatgcacagg gctgacacca

6241 tctgacgcat gatggttcaa agccgtcggc gggaaagcgg ccagatcatc ggcttcgctc

6301 cagtcgatag gat

(1992)). The supernatant was used as a crude enzyme solution.

In this crude solution, ammonium sulfate was saturated up to 50 %, to precipitate proteins which were recovered by centrifugation at 8,000xg for 20 min. The protein mass was dissolved in a 0.02 M phosphate buffer (pH 6.8), followed by dialysis in the same buffer. In this regard, elution was conducted at a rate of 0.5 ml/min through a column (2.5x10 cm) charged with a weak anion exchange resin (DEAE-Toyopearl 650M). In a linear concentration gradient of NaCl from 0 to 0.5 M, the eluate at 0.3 M was collected. The eluate was concentrated and purified followed by Hydroxyapatite column chromatography. After being concentrated, The protein was allowed to precipitate with 20% saturated ammonium sulfate. And finally the concentrate was loaded on a gel filtration column (Superose 12, Pharmacia) to elute a fraction containing a molecular weight of 91,000. The final purification yield was 18.3 fold of the crude enzyme from *Z. mobilis*, with 16.5% of the enzyme recovered in the preparation step (Table 1). The solution was used as a levansucrase solution.

TABLE 1. Summary of levansucrase purification steps from *Z. mobilis*

| Step | Volume (ml) | U total | Protein (mg/ml) | Spec. Act. (U/mg) | Yield (%) | Purifi. Fold |
|---|---|---|---|---|---|---|
| Cell washed | 1,300 | -a | 0.35 | - | - | - |
| 1st $(NH_4)_2SO_4$ | 115 | - | 1.28 | - | - | - |
| Ion-exchange | 38 | 4.35 | 0.57 | 0.21 | 100 | 1.00 |
| Hydroxyapatite | 20 | 2.58 | 0.41 | 0.31 | 65 | 1.52 |
| 2nd $(NH_4)_2SO_4$ | 2 | 0.96 | 0.46 | 1.04 | 21 | 5.07 |
| Superose 12 | 1.5 | 0.72 | 0.13 | 3.75 | 16.5 | 18.3 |

a: could not be determined.